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# Synthesis of Silver Nanoparticles using Ocimum Gratissimum and Cissus Quadrangularis. Assessment of the Antibacterial and Cytotoxic Effect of Nanoparticles

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This study evaluates the antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activity of silver nanoparticles biosynthesized using Ocimum gratissimum (African basil) and Veld grape (Cissus quadrangularis) formulation. Ocimum gratissimum and Cissus quadrangularis mediated AgNPs was studied for antimicrobial activity using agar well diffusion method, time-kill kinetics analysis, and cytotoxic activity using brine shrimp lethality assay. AgNPs showed a maximum zone of inhibition against Pseudomonas sp, E. coli, E. faecalis, and S. aureus of 22, 18, 19, and 17 mm at 100 µg/ml concentration. In the time-kill curve assay, AgNPs showed bacteriostatic activity against all tested pathogens, similar to standard values. As the concentration of nanoparticles increases, their antimicrobial activity increases. 90% live nauplii are observed after 2 days of treatment with 80µg/ml concentration of AgNPs in brine shrimp lethality assay. Results show that AgNPs biosynthesized using O. gratissimum and C. quadrangularis formulation show efficient antimicrobial, antioxidant, and anti-inflammatory activity. Thus, synthesized AgNPs can be further studied and can be utilized in biomedical applications.

**Keywords:** antimicrobial, anti-inflammatory, antioxidant, AgNPs, biomedical applications, cytotoxic effect.

## 1. Introduction

Nanotechnology integrates fundamental characteristics of physical, chemical, and biological sciences and it occurs on a small scale of nanometers. It involves size reduction physically, new chemical bonds and properties are regulated and biological activities are produced at a nano level such as targeted drug delivery. Nanoparticles have one dimension and their size ranges between 1 to 100 nm (Malik et al., 2023). Applications of nanoparticles are cancer diagnosis, MRI contrast enhancement; wound dressing - antimicrobial activity, environmental remediation by adsorption of contaminants onto the surface to NPs, electronics, electrochemical water splitting, and energy generation devices (Khan et al., 2019). Biomedical applications of nanoparticles are in nanomedicine - probing of DNA structure, cancer therapy, diabetes treatment, tissue engineering, protein detection, treatment of neurodegenerative disease, and gene and drug delivery (Sankar et al., 2024). Nanoparticles can be synthesized using bottom-up and top-down techniques. In the top-down approach, bulk material undergoes a series of operations to form nanoparticles. The top-down method is subtractive, producing small and irregularly shaped NPs. Bottom-up approach - is an additive and constructive method, atoms or molecules act as building blocks which are combined to form nanostructures (Tripathy, 2023). Nanoparticles can be synthesized using physical, chemical, and biological methods. Physical and chemical methods utilize toxic chemicals which are harmful to the environment and health. Toxic chemicals used are absorbed on the surface of NPs which can affect their activity in medical applications. Biosynthesized NPs are eco-friendly, reliable, cost-effective, economical, stable nanoparticles that are produced and not noxious. The plant extract has many secondary metabolites which act as capping and reducing agents for NPs synthesis (Ghojavand et al., 2020).

Ocimum gratissimum is a traditional herb, belongs to the Lamiaceae family, widely known as Nimma tulasi and grows in India, South Africa and in tropical regions. It contains various biomolecules such as volatile compounds, flavonoids, polyphenols, tannins and alkaloids like geraniol, eugenol, thymol, citral and linalool which can be extracted from its essential oil which has antifungal activity and used as insect repellent. It is commonly known as lemon or clove basil which is an aromatic shrub (Sharma et al., 2022). Its medicinal activities are antioxidant, anti-inflammatory, anxiolytic, neuroprotective, antinociceptive, anti-anaemic, antimicrobial, analgesic, antiprotozoal, wound healing, neuroprotective, biopesticide, enzyme-inhibitory, anticancer, larvicidal, cytotoxic, hepatoprotective, anti-diabetic, nephroprotective, anti- hypertensive and anti-diarrhoeal activity (Ugbogu et al., 2021). Cissus quadrangularis belongs to the family of Vitaceae, a herbaceous perennial climber which is commonly known as Devil's backbone, Asthisamharaka or Hadjora which is found in hot regions of Sri Lanka India, subtropical and tropical regions of coastal areas. It contains Vitamin C, Vitamin A, flavonoids-quercetin, β-sitosterol, δ-amyrone, δ-amyrin saponins, steroids, flavonoids, triterpenoids, alkaloids, carotene and anabolic steroids (Singh et al., 2021). Pharmacological activities of C.quadrangularis are anticancer, antidiabetic, anti-inflammatory, antimicrobial, anthelmintic, antiobesity, anti-hemorrhoidal, antioxidant and anti-arthritis activity. Traditionally used to treat bowel infections, asthma, broken bones, menstrual disorders, hemorrhoids, burns, boils, hemorrhage, leprosy, chronic ulcer, convulsion, swelling, rheumatic pain, eye disorders, indigestion, hemoptysis, anorexia, dyspepsia, gastritis and anemia (Kumar, 2019).

Silver nanoparticles exhibit antifungal, antibacterial, anti-biofilm, antiviral, anticancer and anti-inflammatory activities. Applications of AgNPs are medical field- wound dressing, wound healing, gum recovery, tooth implant, bone cement, catheter, silver coated medical device, orthopedics- coating of implants and prosthetics, antimicrobial gel, toothpaste, shampoo, soaps, air filters, face mask, hydrogel to prevent biofilm formation and speed up recovery of bone growth (Naganthran et al., 2022). Mechanical property of AgNPs makes it an excellent antibacterial or anticancer agent. Mechanism of cytotoxicity is based on attachment of AgNPs onto microbial cell membrane, increases membrane permeability or alters lipid bilayer, AgNPs penetrates cell intracellularly, cellular toxicity is induced by AgNPs due to ROS and free radicals generation which damages intracellular organelles and signal transduction pathways are altered resulting in apoptosis (Lee & Jun, 2019). ROS species like singlet oxygen, superoxide, epoxyl, hydroxyl and peroxylnitrile produces oxidative stress, leading to diseases like atherosclerosis, inflammation, cancer, neurodegenerative disorder and aging. Antioxidant activity of AgNPs based on capping of NPs with phytochemicals of medicinal plants which makes NPs act as hydrogen donors, reducing agent and singlet oxygen quencher, can be used for treatment of diseases (Mikhailova, 2020).

Silver nanoparticles are synthesized using Ocimum gratissimum and Cissus quadrangularis plant formulation and are assessed for antioxidant, antiinflammatory, antimicrobial and cytotoxic activity using hydrogen peroxide, DPPH, FRAP, ABTS and nitric oxide assay, egg albumin denaturation assay, bovine serum denaturation assay, agar well diffusion method, time-kill curve and brine shrimp lethality assay.

## 2. Materials and methods

### 2.1 Preparation of plant formulation

Fresh leaves of Ocimum gratissimum and stem of Cissus quadrangularis are collected from the herbal garden, rinsed in tap water once and in distilled water and then dried. 5g of both plant samples was weighed using weighing balance. Plant samples are crushed using mortar and pestle, crushed plant formulation was added to 100ml of distilled water. The solution was kept for boiling in the heating mantle in 55°C for 15-20 minutes and the solution was cooled down. The solution was filtered using No.1 Whatman filter paper and aqueous solution was stored for further use.

### 2.2 Synthesis of silver nanoparticles

2mM of silver nitrate was added to 75 ml of distilled water, which acts as a precursor. 25 ml of prepared aqueous formulation (O. gratissimum + C. quadrangularis) was added to prepared 75ml of silver nitrate solution. The mixed solution was kept in the orbital shaker, after 3 and 24 hours, OD value was measured using UV spectroscopy. Observation of color change indicates synthesis of silver nanoparticles.

### 2.3 Antimicrobial activity

#### 2.3.1 Agar well diffusion method:

Sterilized Muller Hilton agar was poured into a petri dish. Wells were made using 9mm polystyrene tips. O. gratissimum and C. quadrangularis plant formulation-mediated AgNPs are taken in varying concentrations - 25, 50, and 100 µg/ml. Plant formulation was used as a control. Pseudomonas sp., E. coli, E. faecalis, and S. aureus species were taken and are individually spread over separate agar plates and the samples were loaded. The plate was kept for incubation and observed after 24 hours. The zone of inhibition reveals the antibacterial efficacy of O. gratissimum and C. quadrangularis-mediated AgNPs.

#### 2.3.2 Time-kill kinetics analysis

1mL of an aliquot of bacterial suspension (Pseudomonas sp., E. coli, E. faecalis, and S. aureus) was added to 9mL of Muller Hinton broth containing 25, 50, and 100 µg of AgNPs. The final microbial concentration is approximately $10^6$ CFU/mL. It was incubated at 37°C with shaking at 200 rpm in time intervals of 0, 1, 2, 3, and 4 hours. The percentage of dead cells was calculated in regular time intervals at a wavelength of 600 nm.

### 2.4 Brine shrimp lethality assay

The cytotoxic effect of AgNPs was assessed using a brine shrimp lethality assay using nauplii eggs. Freshly hatched Brine shrimp - Artemia salina larvae are transferred into 6 wells, with each well containing 10 nauplii. AgNPs are added into each well in varying concentrations of 5, 10, 20, 40, and 80µg/ml. In one well, only live nauplius is taken without any nanoparticles added to it, which serves as a control. Wells are left undisturbed for 24 hours and after 24 hours, the number of live nauplii is noted and data is plotted in a graph. The number of live nauplii is calculated using the following formula: % of live nauplii = (Number of dead nauplii / Number of dead nauplii + number of live nauplii) ×100

## 3. Results:

### 3.1 Preparation of silver nanoparticles using plant formulation

Figure 1: Preparation of Silver nanoparticles using O. gratissimum and C. quadrangularis a) Leaf extract of O. gratissimum, b) Plant extract of C. quadrangularis, c) O. gratissimum and C. quadrangularis plant formulation in heating mantle, d) O. gratissimum and C. quadrangularis plant formulation, e) Silver nitrate solution, f) silver nitrate solution was mixed with plant extract for synthesizing AgNPs and g) 24 hours after the synthesis of AgNPs

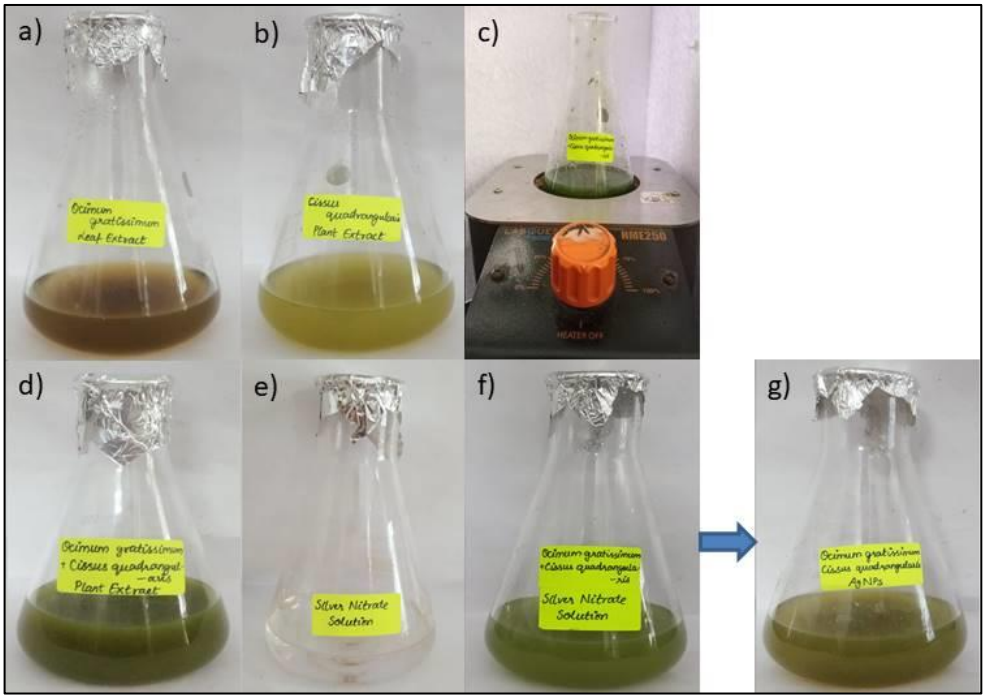



Figure 1

3.2 Antimicrobial activity:

3.2.1 Agar well diffusion assay:

Antibacterial activity of biosynthesized AgNPs was evaluated by assessing its zone of inhibition produced against Pseudomonas sp., Escherichia coli, Enterococcus faecalis and Staphylococcus aureus species. As the concentration of NPs increases, its zone of inhibition against the bacteria increases. At the highest tested concentration of 100 µg/mL, the maximum zones of inhibition exhibited by AgNPs against the above-mentioned pathogens are 22, 18, 19, and 17 mm as shown in figure 2. The graphical representation of antimicrobial activity against wound pathogens was represented in Figure 3.

Figure 2: Zone of inhibition produced by biosynthesized AgNPs against a) Pseudomonas sp., b) Escherichia coli, c) Staphylococcus aureus and d) Enterococcus faecalis

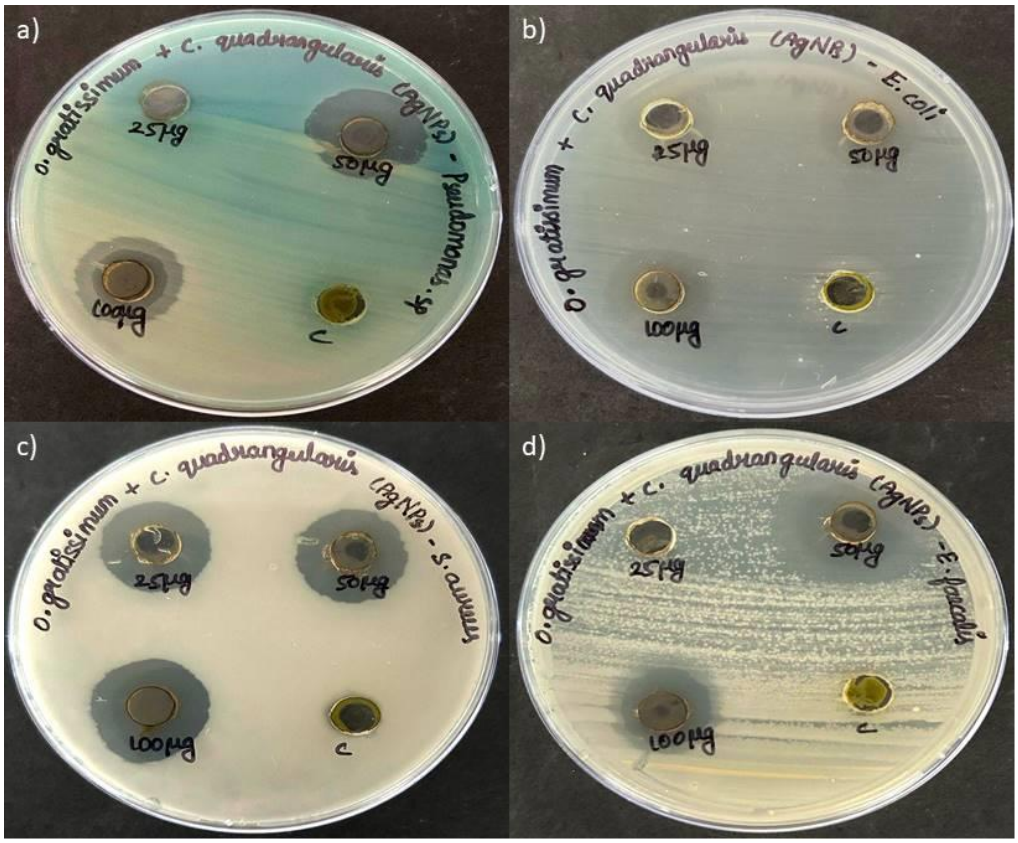



Figure 2

Figure 3: Antimicrobial activity of O. gratissimum and C. quadrangularis mediated AgNPs against pathogens assessed using agar well diffusion assay.

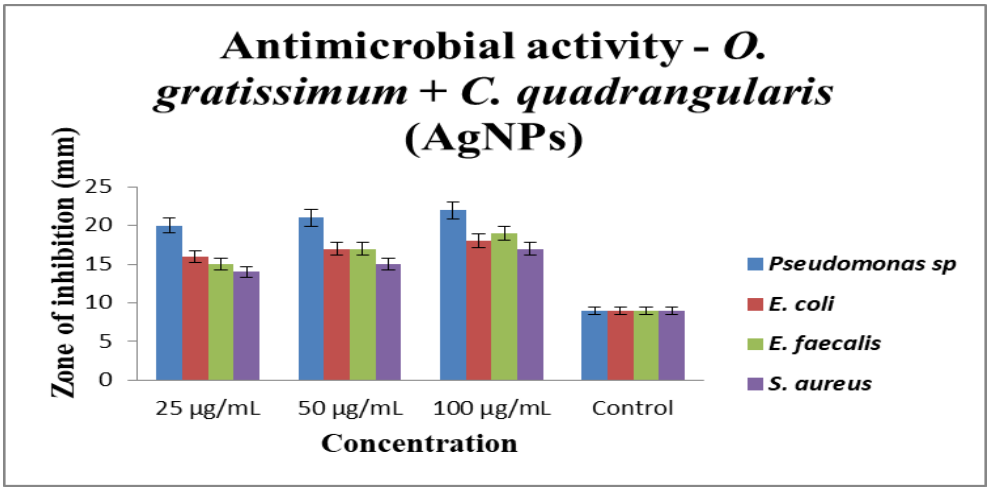
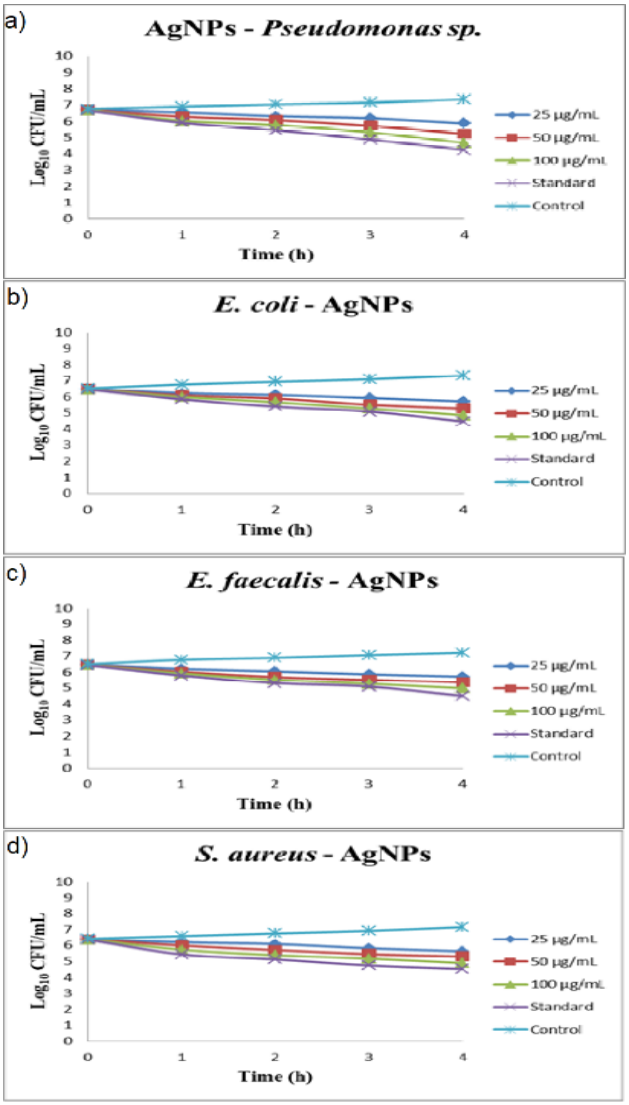



Figure 3

### 3.2.2 Time-kill kinetics analysis:

The $Log_{10}$ CFU value of Pseudomonas sp. decreases at the highest tested concentration of 100 µg/mL of AgNPs in the period of 4 hours which shows bacteriostatic activity. The values are similar to those of standard $Log_{10}$ CFU. In 100 µg/mL concentration of AgNPs, the $Log_{10}$ CFU of E. coli reduces at a period of 4 hours which shows bacteriostatic activity of AgNPs. The values are similar to that of the standard. At the highest tested concentration of 100µg/mL, the Log10 CFU values of E. faecalis decrease in a time span of 4 hours, which shows the bacteriostatic activity of AgNPs. At 100 µg/mL concentration of AgNPs, $Log_{10}$ CFU values of S. aureus decrease in a time span of 4 hours which shows bacteriostatic activity of biosynthesized AgNPs as shown in Figure 4.

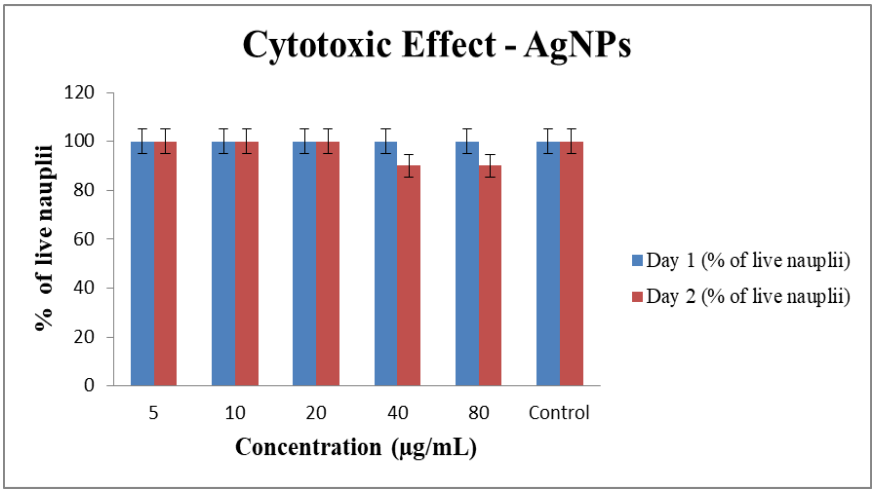
Figure 4: Time kill curve analysis of silver nanoparticles against wound pathogens.



3.3 Cytotoxic activity - Brine shrimp lethality assay:

Biosynthesized silver NPs are tested on brine shrimp (Artemia salina) nauplii in concentrations of 5, 10, 20, 40 and 80 µg/ml. On day 2, the percentage of live nauplii observed in the tested concentrations of AgNPs are 100, 100, 100, 90 and 90% as shown in Figure 5.

Figure 5: Cytotoxic activity of AgNPs assessed using brine shrimp lethality assay




**4. Discussion:**

Results show that the maximum zone of inhibition exhibited by AgNPs was observed at the highest tested concentration of 50 µg/mL with values of 22 mm - for Pseudomonas sp, 18mm - for E. coli, 19 mm- for E. faecalis and 17mm - for S .aureus in agar well diffusion method. Previously done studies show that - 50µl of Spinacia oleracea leaf extract-mediated AgNPs are tested against E. coli, P. aeruginosa, and S. aureus. Their respective zone of inhibition values are 27, 32, and 31 mm (Mekky et al., 2021). Abelmoschus esculentus aqueous flower extract mediated AgNPs (AME-AgNPs) are taken in concentrations ranging between 5 to 100µg/mL. At the highest tested concentration of 100µg/mL, AME-AgNPs showed zone of inhibition of 11 mm against P. aeruginosa, 13mm against E.coli and 13mm against S. aureus (Devanesan & AlSalhi, 2021). Areca catechu extract-mediated AgNPs are taken in varying concentrations of 11.25, 22.5, 45, 90, 180 and 360 µg/mL. The zone of inhibition exhibited by AgNPs against E. faecalis in concentrations of 90 and 360µg/mL are 10.5 ± 0.5 and 12.0 ± 0.9 mm. For the same concentrations of AgNPs, the zone of inhibition against P. aeruginosa is 17.5 ± 0.8 and 19.8 ± 1.3 mm respectively (Choi et al., 2021).

O. gratissimum and C. quadrangularis mediated AgNPs in time-kill curve assay showed a decrease in $Log_{10}$ CFU values of Pseudomonas sp from 6.68 to 4.66, from 6.49 to 4.87 for E. coli, from 6.46 to 4.99 for E. faecalis and from 6.37 to 4.88 for S. aureus in a time span of 4 hours which shows the bacteriostatic activity of AgNPs. The values of AgNPs are similar to that of the standard. Previously done studies show that after 120 minutes of exposure, MIC and 2 MIC concentration of Hypericum perforatum mediated AgNPs reduced $10^3$-fold of CFU/mL of S. aureus and after 240 minutes of exposure, all the bacteria are killed which

confirms the bactericidal activity of AgNPs (Alahmad et al., 2022). Stenocereus queretaroensis fruit peel mediated AgNPs of 2×MIC concentration showed bactericidal activity after 2 hours of exposure in E. coli, P. aeruginosa and S. aureus. It showed reduction in value (2.34) for E. coli, 2.46 for S. aureus and 1.63 logs for P. aeruginosa. Bacteriostatic activity was seen in E. coli and P. aeruginosa in 1/2 × MIC concentration of AgNPs (Padilla-Camberos et al., 2021). 1× MIC (0.125mg/mL) concentration of S. polyanthum leaf extract mediated AgNPs (SP-AgNPs) showed reduction Log10 CFU from 7.07 to 6.01 in E.coli after 4 hours of exposure and showed bacteriostatic activity. 2×MIC (0.016 mg/mL) and 4×MIC (0.032 mg/mL) concentration of SP-AgNPs, showed bactericidal effects in S.aureus after 4 and 2 hours of incubation respectively (Khan et al., 2023). 90% of live nauplii are observed after 2 days of treatment with biosynthesized AgNPs in brine shrimp lethality assay. Similarly done research shows that freshly synthesized garlic mediated AgNPs (FGAgNPs) and GAgNPs exhibited mortality of larvae of 70% and 65% at highest tested concentration of 200µg/mL (Andleeb et al., 2020). Chitosan functionalized silver nanoparticles (CS-AgNPs) showed low toxicity with survivors ranging from 93.3 ± 1.32 to 11.2 ± 3.83% for the concentrations ranging from 100 to 1000 µg/ml (Asghar et al., 2020). At highest tested concentration of 1µg/mL, AgNPs synthesized using pomegranate peel extract (P-AgNPs), quercetin (Q-AgNPs) and gallic acid (GA-AgNPs) showed mortality of larvae of 100%, 88.5% and 89.2 % respectively (Saad et al., 2021).

## 5. Conclusion:

O. gratissimum and C. quadrangularis mediated AgNPs showed efficient antioxidant, antimicrobial and anti-inflammatory activity. Dose based activity of nanoparticles was seen in all the activities. 90% of live nauplii are observed at concentration of 80µg/ml of AgNPs after 2 days of exposure, shows that biosynthesized AgNPs are safe and less toxic to cells. Thus further research is needed on AgNPs to assess its activity for safety and further application in therapeutics.

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